# THE EPIBRANCHIAL PLACODES OF SQUALUS ACANTHIAS.

(Fifty-two Figures and Two Tables.)

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The study of the epibranchial placodes of Squalus Acanthias was undertaken with a view to determining whether this type displays the characteristics with regard to contribution of cells to the visceral ganglia of the gill region by the corresponding placodes, which were described by Landacre ('10) in the catfish and ('12) Lepidosteus osseus.

In the catfish, this author described a contribution en masse, of placodal cells to Gang. VII, IX and X, by the corresponding placodes and concluded that the cells from these sources gave rise to gustatory or special visceral fibers.

In Lepidosteus, it was shown that the method of contribution begins by active proliferation of cells of the ectoderm, thus forming the placode. This process is followed by a contact between the ganglia and the placodes, due to a mesial migration resulting in the fusion of the placodal cells with the general visceral components of VII, IX and X. In Rana, Landacre and McLellan ('12) were unable to distinguish a definite gustatory division in the ganglia of the 8 mm. larva probably on account of the more rapid development in this form; but these authors describe the behavior of the epibranchial placodes as similar to that observed in Lepidosteus and Ameiurus, and also describe well defined placodes in the stages earlier than the 8 mm. larva.

The material used consisted of a 20 mm. shark embryo cut into sections 10 microns thick, stained with Delafield's hematoxylin and counter-stained with Orange G. An 18 mm. embryo, subjected to the same technic was used for comparison. All drawings were made with a camera lucida in magnifications of 50x and 620x respectively, and reduced to one-third the original size in reproduction.

Owing to the difficulty of securing successive stages of Squalus at close intervals, this study is based upon a comparison of all the epibranchial placodes in one specimen. The placodes

appear in other forms, as do the branchial clefts, in serial order from anterior to posterior, and it seemed probable that the various stages in development of the placodes of the VII, IX and of the four divisions of the X nerve would present the same kind of evidence that could be secured by a study of one placode, such, for instance, as that of the IX nerve, through a series of embryos of successively older stages.

In determining the relation of a visceral ganglion to the ectoderm, it is necessary to distinguish carefully, the following ectodermal thickenings: (a) the lateral line placode; (b) the thickening of the ectoderm at the point where the entodermal, pharyngeal pocket joins the ectoderm; (c) the ectodermal thickening extending anterior and posterior to (b); (d) thickenings of the epithelium of the entodermal, pharyngeal pocket which, after the gill slits are open, are continuous with the corresponding ectodermal thickenings (b).

A comparison of the 20 mm. and 18 mm. stages indicates that, of the ganglia in question, the VII undergoes the earliest development, hence, in the later stages, presents a more highly developed condition, and here, the placode is very marked and easily distinguishable from the thickenings associated with the gill clefts.

The placodes are characterized by a thickening of the ectoderm, by irregular arrangement of the cells, and by the presence of numerous mitotic figures, indicating rapid cell proliferation.

In the cases where actual cell contribution is observed, this activity takes place almost uniformly by migration of the cells from the placode toward the ganglion, followed by metamorphosis as follows; the cells on the mesial border of the migrating mass exhibit the characteristic darkly-staining, granular nuclei described by Landacre in Lepidosteus; in some cases, also, smaller size of the nuclei than those of the placodal cells that have not migrated, or of the cells of the visceral components, though this is by no means as constant as in Lepidosteus, and in fact, was not clearly marked in any case except Gang. IX.

Table I shows the ratio between the length of the area of contact between the placodes and the ganglia, and the total length of the ganglia themselves. It will be seen from this that the lengths of the areas of contact increase as we pass

posteriorly while the lengths of the ganglia diminish. Thus, there is, progressively, a greater extent of contact between the placodes and ganglia in proportion to the length of the ganglia. This is evidence of the greater maturity of the more anterior ganglia, which is in accord with the general law of antero-posterior differentiation.

TABLE I.

(Showing length of ganglia and contact area in 20 mm. embryo.)

| GANG. | LENGTH OF GANGLIA | LENGTH OF CONTACT AREA | RATIO |
|---|---|---|---|
| VII | 340 microns | 10 microns | 1–34. |
| IX | 190 " | 40 " | 1–4.75 |
| $X_1$ | 270 " | 90 " | 1–3. |
| $X_2$ | 130 " | 90 " | 1–1.46 |
| $X_3$ | 150 " | 120 " | 1–1.25 |
| $X_4$ | 210 " | 160 " | 1–1.4 |

Table II shows the length of the area of contact in the 18 mm. stage. The development has not progressed to the degree seen in the older stage and, consequently, there is less difference in the lengths of the contact areas in the younger stage than in the 20 mm. stage. The placodal contributions are proportionately much larger when compared to the size of the general visceral portions than in the older series. This is evidence that the total absence of the darkly-staining cells in the 22 mm. embryo, examined in connection with other work in progress in the department, may be interpreted as meaning, only, that these cells, in the latter case, have undergone complete metamorphosis and mersion with the general visceral cells so that they do not stain differently. This is also the probable explanation of the failure to distinguish them in Rana (Landacre and McLellan '12), since this form undergoes relatively more rapid development and the process was completed in the earliest stages studied.

TABLE II.

(Showing length of contact area in 18 mm. embryo.)

| GANG. | LENGTH OF CONTACT AREA |
|---|---|
| VII | 180 microns |
| IX | 160 " |
| $X_1$ | 100 " |
| $X_2$ | 90 " |
| $X_3$ | 80 " |
| $X_4$ | 100 " |

### Gang. VII.

Since this ganglion is the earliest to develop, there is, in the 20 mm. stage, a relatively small number of cells showing the characteristic distinguishing features of placodal cells. The ganglion is 340 microns long, but for only 10 microns or through one section, was there any actual contact with the ectoderm, while in the 18 mm. stage there is contact through 18 sections or 180 microns (Tables I and 11). In the 22 mm. stage, there is no contact and no cells which show the characteristic nuclei to a degree sufficient to permit of their being distinguished from the general visceral cells.

In the 20 mm. embryo, most of the ganglion lies anterior and dorso-mesial to the anterior extremity of the first true gill cleft, and the point of contact is just opposite the anterior end of the cleft. There are a number of mitotic figures in the placode and the migrating cell mass, showing that the processes of proliferation and metamorphosis are not yet completed.

The placode is an extensive thickening of the skin and at one point (Fig. 1 and 8), there seems to be a tendency to lamellation. The placode lies quite free and distinct from the thickening of the ectoderm which accompanies the opening of the gill cleft (c). Throughout most of its extent, the ganglion shows a well defined, encapsulated outline, which condition indicates maturity since it is not present to so great a degree in the more posterior ganglia.

The ventro-lateral lateralis component (Fig. 1 to 9, V. L. VII), constitutes the dorsal portion of the ganglionic mass and extends several sections posterior to the limits of the visceral portion.

### Gang. IX.

This ganglion is 190 microns in length and the contact area occupies 40 microns of this length, giving a ratio of 1–4.75 between the total length and the length of the area of contact. The point of contact is approximately opposite the middle of the gill cleft and toward the posterior end of the ganglion. In the 18 mm. stage, the contact area is 160 microns long and so is not much smaller in extent than the contact area of VII in the same stage (Table II).

The placodal thickening of the ectoderm is not so marked as in VII, but the irregular arrangement of the cells, the presence of mitotic figures and the tendency to lamellation of the placodal mass (Fig. 14 and 15) are evidences of the integrity of the mass, which is quite distinct from the lateralis placode and, toward the posterior end of the ganglion, from the gill cleft thickenings also. The outline of the general visceral portion of the ganglion is maintained for several sections posterior to the point of contact (Fig. 10) with the placodal mass, and the boundary line between this and the placodal mass is quite distinct and intact at some points (Fig. 10, 11, 12 and 15).

In the mass of contributed cells, as well as in the placode, there are numerous mitotic figures and there is, in every section, a large group of undifferentiated cells (Fig. 11 to 15), lying near the ectoderm, except near the anterior extremity of the ganglion. Mesial to this mass is an area of cells with much smaller, dark, granular nuclei, representing a stage of incomplete metamorphosis (Fig. 10 to 16, S. V. VII). The boundary between this and the general visceral mass is quite abrupt and distinct in most sections, but in some there is evidence of fusion with the latter mass (Fig. 13). These facts are presented as evidences of the active state of proliferation and metamorphosis.

The process of contribution to the ganglion does not persist posterior to the point of disappearance of the general visceral portion, though the placode persists several sections posterior to both.

It will be seen from these conditions, that Gang. IX presents a much less mature condition than Gang. VII, which is to be expected from the evidences already presented (Tables I and II).

### Gang. X.

The main mass of the ganglion does not come into contact with the ectoderm except at the extreme posterior extremity; instead, it gives off four branchial ganglionic masses which extend ventro-lateral something after the manner of the fingers of a hand, and come into contact with the ectoderm of the third, fourth, fifth and sixth gill bars respectively. The length of each branchial division is measured from the point of its complete separation from the main ganglionic mass.

The length of the first branchial ganglion of X, is 270 microns and the area of contact is 90 microns in length and situated toward the posterior end of the ganglion. This gives a ratio of 1–3 between the length of the contact area and the total ganglion length (Table I). In the 18 mm. stage, the length of the area of contact is 100 microns (Table II).

The oval outline of the ganglion, as seen anterior to the point of contact, persists posterior to the first section in which contact is seen (Fig. 22), but finally becomes indented by contact with the placodal mass so that the lateral curve is lost (Fig. 23 and 24), but the boundary between the general visceral and placodal components is quite distinct and persists throughout the entire length of the contact. The contact occurs toward the posterior end of the gill cleft and is directly mesial to the external aperture of the cleft. On account of its proximity to this structure, it is impossible to distinguish the placode from the other ectodermal thickenings associated with the gill clefts.

In Fig. 22, the cells with dark, granular nuclei lie in contact with the placode and there are no mitotic figures, showing that the processes of contribution and metamorphosis are slower in this region and so, more nearly complete toward the anterior end of the contact. This is true of all ganglia. In the more posterior sections (Fig. 23 and 24), there is evidence of more active proliferation, since there are large masses of undifferentiated cells to be seen, which have probably become detached en masse, lying near the ectoderm. The smaller size of the nuclei of the placodal cells is not so marked as in Gang. IX.

The second branchial ganglion of X presents a different arrangement from the first, in that the point of contact is with the entodermal evagination from the pharynx which enters into the formation of the gill cleft, at the anterior end; toward the posterior end, the contact is with the ectoderm at a point dorso-mesial to the external aperture of the cleft. The branchial ganglion is entirely free from the main ganglionic mass of X several sections anterior to the anterior end of the gill cleft.

The length of the ganglion is 130 microns and the length of the contact area is 90 microns, giving a ratio of 1–1.46 between the contact length and the total ganglion length. In the 18 mm. larva, the length of the contact area is 90 microns, also, though the length of the ganglion is not so great as in the older stage (Tables I and II).

The distinction between the placode and the gill cleft thickenings is difficult to determine, at least, in the more anterior sections, though the irregular arrangement of the cells of the ectoderm and the presence of a few mitotic figures are evidences of proliferation. In the first section in which contact is seen, the mass of contributed cells is quite large and persists posterior to the point of disappearance of the visceral portion, so that the placodal portion lies well toward the posterior end of the ganglion, most of which lies anterior to the middle of the gill cleft.

The oval outline of the visceral component persists after contact (Fig. 30) and the boundary between this and the placodal component is quite distinct, even posterior to the point at which the lateral curve of the visceral mass becomes indented by contact with the placodal mass (Fig. 31). In the more posterior sections (Fig. 32 and 33), there is fusion between the two components to such an extent that the boundary is not so distinct. There is evidence of rapid contribution in the presence of a large mass of undifferentiated cells near the ectoderm (Figs. 30, 31 and 32). The metamorphosing cells possess nuclei but slightly smaller in size than those of the other cells of the ganglion.

In the third branchial ganglion of X, also, the most anterior contact is with the entodermal gill pocket from the pharynx instead of with the ectoderm (Fig. 34, 35, 36 and 37). The length of the ganglion is 150 microns and that of the contact area, 120 microns, giving a ratio of 1–1.25 between the length of the area of contact and that of the total ganglion. In the 18 mm. larva, the length of the contact area is 80 microns, showing that the process of contribution has probably not progressed to so great a degree in the 20 mm. stage as it has in the VII and IX ganglia in the younger stage (Tables I and II).

In the more anterior regions, it is impossible to distinguish between the placode and the gill cleft thickenings but, in the more posterior regions, the distinction is quite clear (Fig. 39, 40 and 41). Proliferation and metamorphosis are evidently going on quite rapidly throughout the entire length of the contact and the placodal mass persists in considerable size to the posterior end of the ganglion. There is no appreciable difference in size between the nuclei of the metamorphosing cells and those of the neighboring cells but the dark stain and

the granular appearance are in evidence throughout. There is evidence of active proliferation in the placode, though metamorphosis is evidently not proceeding so rapidly, since the mass of metamorphosing cells is quite large in proportion to the size of the mass of undifferentiated cells. There is no distinct boundary line between the placodal and general visceral components, showing that fusion between the two is quite complete. The placodal mass does not persist to the posterior extremity of the ganglion but seems to occupy a position about the middle of this structure.

In the fourth epibranchial of division X, the form and position of the ganglionic mass are such as to make the contributed mass probably appear larger than it actually is. The length of the ganglion is 210 microns and that of the contact area is 180 microns, giving a ratio of 1–1.4 between the contact area and the total length (Table I). In the 18 mm. embryo, the length of the contact area is 100 microns. This is further evidence of a lesser degree of maturity in the more posterior ganglia.

The point of contact lies dorso-mesial to the middle of the external aperture of the gill cleft (Fig. 42 to 50). The placode is easily distinguishable from the other ectodermal thickenings throughout the entire length of the ganglion (Fig. 43, 44, 45, 47 and 48). The general visceral portion of the ganglion does not maintain its outline after contact with the mass of contributed cells and there is such complete fusion between the two masses that a definite boundary is not discernable except in Fig. 49. The presence of mitotic figures, the complete fusion between the general visceral and placodal masses and the large size of the latter, indicate very rapid proliferation, while the large size of the mass of incompletely metamorphosed cells as compared to the size of the mass of undifferentiated cells, indicates comparatively slow metamorphosis.

In Fig. 45 and 46 there may be seen a constriction in the mass of contributed cells which later results in complete separation between the ganglion and the placode (Fig. 47, 48, 49 and 50), leaving a large mass of cells attached to the placode. This mass, in some sections, shows a tendency to lamellation (Fig. 49 and 50). Posterior to the point of complete separation of the ganglion from the placode, the contributed mass is relatively much smaller than the general visceral portion.

There is another point of contact between the ectoderm and the main ganglion of X in the post-branchial groove where there is an appreciable thickening of the skin and a definite enlargement of the ganglion, also some evidence of contribution of ectodermal cells to the ganglion, but no separate epibranchial division.

## Summary.

1. The epibranchial placodes of Squalus Acanthias arise as proliferations of the ectoderm about the middle and dorsal region of the corresponding branchial clefts.

2. Contribution of cells by the placodes to the visceral ganglia is by proliferation and mesial migration, the cells coming into contact with the caudal extremity of the corresponding ganglia.

3. With but two exceptions, the placodes are easily distinguishable from the other ectodermal thickenings in the same regions.

4. The placodal cells, in the course of migration, undergo a process of metamorphosis, during which the nuclei become darker and more finely granular, and in Gang. IX, smaller in size. In the older ganglia these migrating masses of placodal cells are completely fused with the general visceral masses and the cells of the two components are indistinguishable from each other.

5. There is a general similarity in behavior between the placodal cells in the shark and those of other forms in which this process has already been described.

6. The order of maturity of the epibranchial ganglia is from anterior to posterior, in progressive stages.

LITERATURE CITED.

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**Landacre, F. L.** 1912. The Epibranchial Plocodes of Lepidosteus osseus and their Relation to the Cerebral Ganglia. ibid. Vol. XXII, No. 1, p. 1.

**Landacre, F. L.,** and **McLellan, Marie.** 1912. The Cerebral Ganglia of The Embryo of Rana pipiens, ibid, Vol. XXII, No. 5, p. 461.

## EXPLANATION OF FIGURES.

All drawings were made from the posterior surfaces of 10 micron sections, with a camera lucida. Outline drawings were magnified 50x; high power drawings were magnified 620x. All figures were reduced to one-third the original size in reproducing. The sections are numbered serially from anterior to posterior, showing the relations of the sections used in the drawings.

## KEY TO ABBREVIATIONS.

Aud.—Auditory vescicle.
Ec.—Ectoderm.
G. C.—Gill cleft.
G. V. VII—General visceral portion of the seventh ganglion.
G. V. IX—General visceral division of the ninth ganglion.
G. V. $X_1$, $X_2$, $X_3$, $X_4$—General visceral divisions of the first, second, third and fourth epibranchial ganglia of the tenth nerve.
G. X—The main ganglionic mass of the X nerve.
L. IX—Lateral line portion of Gang. IX.
L. X—Lateral line portion of Gang. X.
L. Pl.—Lateral line placode.
M.—Metencephalon.
Pl.—Placode.
S. V. VII—Special visceral portion of Gang. VII.
S. V. IX—Special visceral portion of Gang. IX.
S. V. X—Special visceral portion of the main ganglionic mass of the X nerve.
S. V. $X_1$, $X_2$, $X_3$, $X_4$—Special visceral divisions of the first, second, third and fourth epibranchial ganglia of the X nerve.
V.—Blood vessel.
V. L. VII—Ventral lateral line division of Gang. VII.

## PLATE XXIII.

Fig. 1. Sec. No. 216. Gang. VII, showing the placodal (Pl) thickening of the ectoderm at a point opposite the ganglion. The placodal contribution is small at this point and is characterized by the presence of cells with darkly staining nuclei; there are a number of mitotic figures, indicating active proliferation. Actual contact between the ganglion and the ectoderm is not present in this section.

Fig. 2. Sec. No. 222. G. VII. The distinguishable portion of the placodal contribution is much larger than in the preceding figure. There is a contact between the ganglion and the ectoderm, and the fact that the most external cells of the contributed mass do not show the characteristic dark nuclei, indicates active contribution of cells by the placode. The most recently contributed cells have not yet undergone the stage of metamorphosis seen in those that have migrated farther into the body of the ganglion.

Fig. 3. Sec. No. 224. G. VII. In this section, there is a reduction in the comparative size of the placodal component, absence of contact and a reduction in the number of mitotic figures, from the number in previous sections.

Fig. 4. Sec. No. 227. G. VII. There is still further reduction in the comparative size of the placodal component and a comparatively large area of undifferentiated cells on the external portion of the ganglion near the ectoderm.

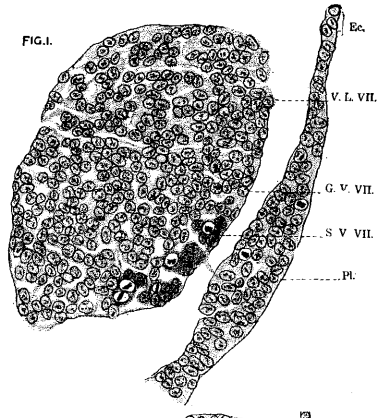
Fig. 5. Sec. No. 231. G. VII. There is no evidence of actual cellular contact between the placode and the ganglion and, while still very near the placode, there is no evidence of recently contributed, undifferentiated cells on the lateral periphery of the ganglion.

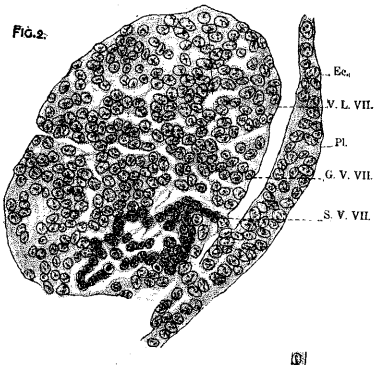
Fig. 6. Sec. No. 233. G. VII. The dorsal portion of this section and the sections already described, constitute the lateral line component of VII (V. L. VII), the lower portion, the general visceral component; the latter, in this section, occupies an area of about the same extent as the placodal component, though this is reduced from the extent displayed in the previous section. There is still some active proliferation as evidenced by the presence of mitotic figures, not all of which are in focus at this level.

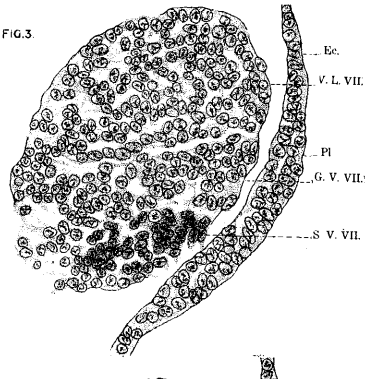
Fig. 7. Sec. No. 237. G. VII. The body of the entire ganglion is not so clearly defined at this point as in the more anterior and more mature portions. The placodal thickening is not greatly reduced and there is evidence of very active cell proliferation, though actual continuity of cellular elements is doubtful.

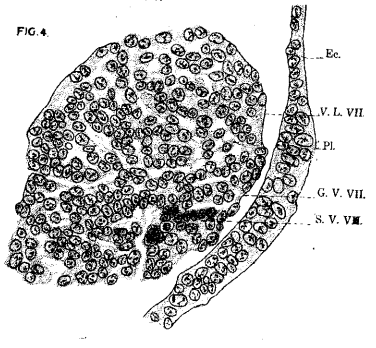
Fig. 8. Sec. No. 216. G. VII. An outline drawing of the same section as Fig. 1, showing the general relations of the area. The lateralis component is relatively smaller than the general visceral. The relation of the entire ganglion and the placode to the first gill cleft is clearly seen. There can be no doubt as to the independence of the placodal thickening from that situated at the opening of the gill cleft.

Fig. 9. Sec. No. 224. G. VII. An outline drawing of the same section as Fig. 3. The branchial cleft has deepened and the gill cleft thickening of the ectoderm is consequently carried farther away from the placodal thickening, which still maintains the form and size displayed in the intervening sections, as well as in those situated more posteriorly.

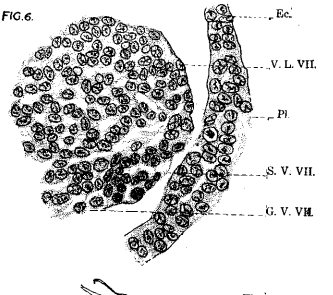


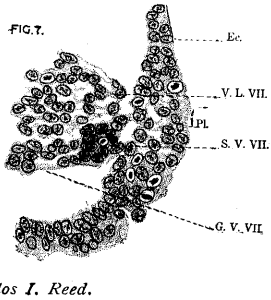



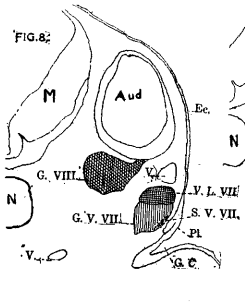



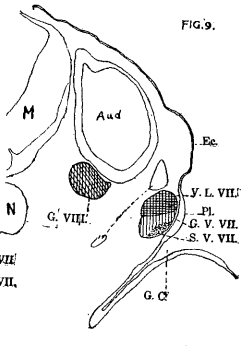





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## PLATE XXIV.

Fig. 10. Sec. No. 278. G. IX. A high power drawing of the most anterior section of Gang. IX in which there is contact with the ectoderm. The placodal thickening is quite marked and there is some evidence of active proliferation in the placodal area. The cells of the placodal component are smaller than those of the general visceral portion. There are a few cells near the ectoderm which have evidently been recently contributed from the placode and have not yet undergone metamorphosis.

Fig. 11. Sec. No. 280. G. IX. In this section, the smaller size of the nuclei of placodal cells is quite marked. The outline of the general visceral component is easily distinguishable throughout part of the extent of the contact with the placodal portion. Here again, may be seen a small mass of undifferentiated placodal cells lying near the ectoderm.

Fig. 12. Sec. No. 281. G. IX. In this section, the metamorphosing cells are completely surrounded by undifferentiated cells. The presence of mitotic figures indicates active proliferation.

Fig. 13. Sec. No. 282. G. IX. The extent of the contact with the ectoderm is greater than in the previous section. Proliferation must be going on more rapidly than metamorphosis, since the mass of undifferentiated cells near the ectoderm is larger and shows the cells massed more closely and tending to form lamellae.

Fig. 14. Sec. No. 284. G. IX. In this section, proliferation is very rapid as evidenced by the large mass of undifferentiated cells and the comparatively narrow field of placodal cells which have undergone metamorphosis. The marginal extent of contact is shorter than in the more anterior sections.

Fig. 15. Sec. No. 286. G. IX. The contributed portion of the ganglion is very large in proportion to the size of the general visceral ganglion. Several mitotic figures may be seen and there is a well marked margin of undifferentiated cells. The placodal thickening is dorso-lateral to the contact area which is still shorter in extent than in Fig. 14.

Fig. 16. Sec. No. 289. G. IX. The extensive placodal thickening still persists and active proliferation is still going on.

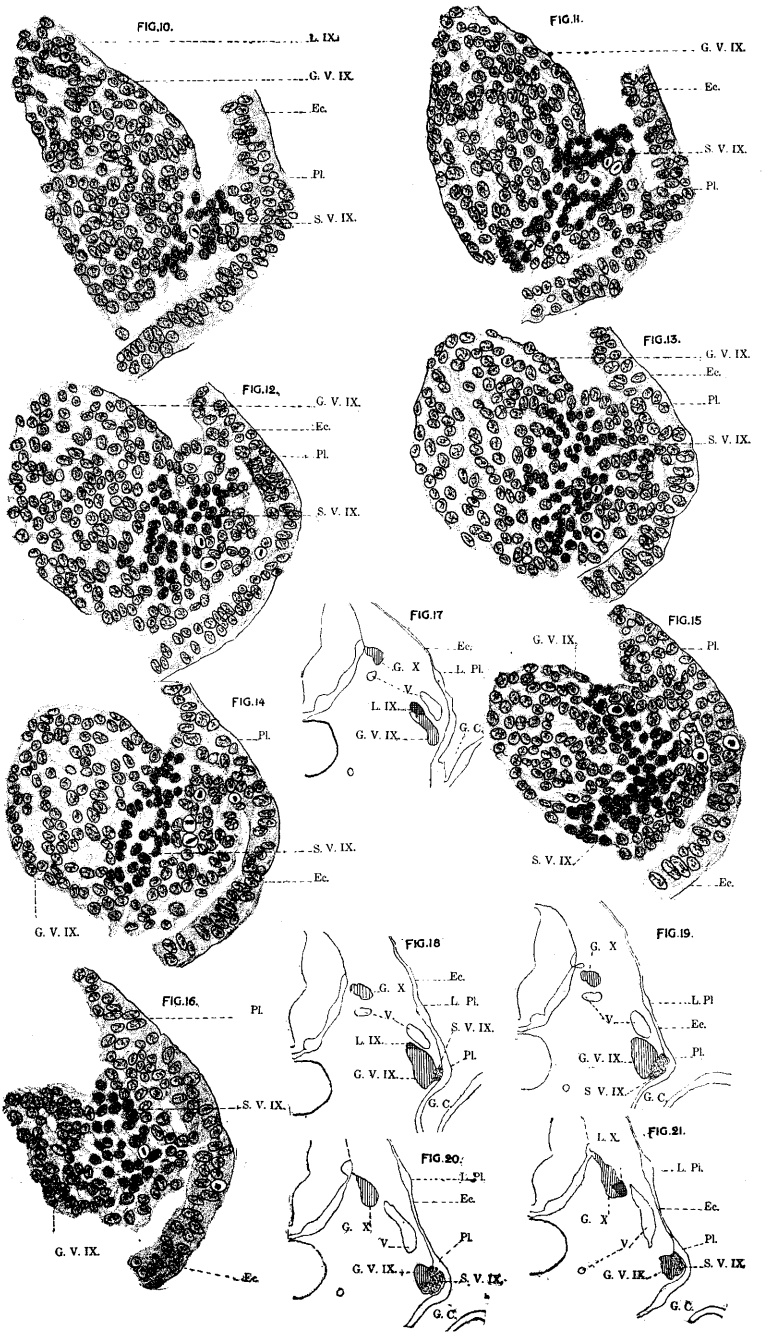
Fig. 17. Sec. No. 275. G. IX. This section shows the relation of the lateralis and visceral components anterior to the point of contact. It is, here, impossible to distinguish the placodal thickening from that accompanying the gill cleft opening, which lies ventral to it.

Fig. 18. Sec. No. 278. G. IX. An outline drawing of the same section as Fig. 10. The lateral line placode is very distinct from the epibranchial placode. The epibranchial placode, since the gill cleft is open, is easily distinguishable from the gill cleft thickenings of the ectoderm.

Fig. 19. Sec. No. 280. G. IX. An outline drawing of the same section as Fig. 11, showing the relation of the contributed mass to other structures.

Fig. 20. Sec. No. 287. G. IX. An outline drawing one section posterior to Fig. 15, showing the dorso-lateral point of contact.

Fig. 21. Sec. No. 296. G. IX. Showing the appearance of the lateralis component of Gang. X, the great thickness of the lateral line placode, and the relative size of the placodal and general visceral portions of Gang. IX.

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## PLATE XXV.

Fig. 22. Sec. No. 320. G. $X_1$. Showing the contact point just mesial to the external opening of the gill cleft. There are no mitotic figures in the contributing area, but there may be seen a very definite zone of metamorphosing cells along the lateral border of the ganglion.

Fig. 23. Sec. No. 324. G. $X_1$. Two points of contact are seen and proliferation is going on quite rapidly, from the fact that there is a large mass of larger and more clearly stained cells between the two points of contact and external to the metamorphosing zone. The outline of the visceral portion of the ganglion is only fairly distinct.

Fig. 24. Sec. No. 326. G. $X_1$. The mass of metamorphosing cells is almost surrounded by undifferentiated cells. This, together with the presence of mitotic figures, indicates active proliferation from the placodal area.

Fig. 25. Sec. No. 323. G. $X_1$. An outline drawing of the section anterior to Fig. 23, showing the general topography and the relation of the ganglion to the gill cleft and the placode, the latter being indistinguishable from the ectodermal thickening accompanying the opening of the gill cleft.

Fig. 26. Sec. No. 345. G. X. This section shows the relation of the main ganglion to the ectoderm and the gill groove. The second epibranchial ganglion of X, G, V, $X_2$, is seen partly constricted off from the main ganglionic mass. A lateral line nerve is seen leading to the easily distinguished lateral line organ.

Fig. 27. Sec. No. 350. In this figure, the epibranchial ganglion is seen lying close to a thickening in the epithelial lining of the pharyngeal gill pouch. This thickening is continuous, in later sections, with the placodal thickening of the ectoderm, posterior to the point at which the gill cleft is entirely open.

Fig. 28. Sec. No. 351. The endothelial thickening persists and is nearer the external aperture of the gill cleft.

Fig. 29. Sec. No. 352. The endothelial thickening is more clearly defined than in the previous section.

Fig. 30. Sec. No. 352. G. $X_2$. A high power drawing of the same section as in the preceding figure, showing proliferation and a mass of cells with darkly stained nuclei which are not appreciably smaller than those of the cells of the general visceral ganglion. The oval outline of the general visceral portion and the boundary between this and the placodal portion are clearly seen.

Fig. 31. Sec. No. 353. G. $X_2$. Here the placodal thickening is clearly seen and there is much evidence of active proliferation.

Fig. 32. Sec. No. 357. G. $X_2$. The placodal thickening is still seen but the extent of contact with the ganglion is slight. The placodal cells constitute a much larger mass than the general visceral cells.

Fig. 33. Sec. No. 357. G. $X_2$. An outline drawing of the same section as shown in Fig. 32.

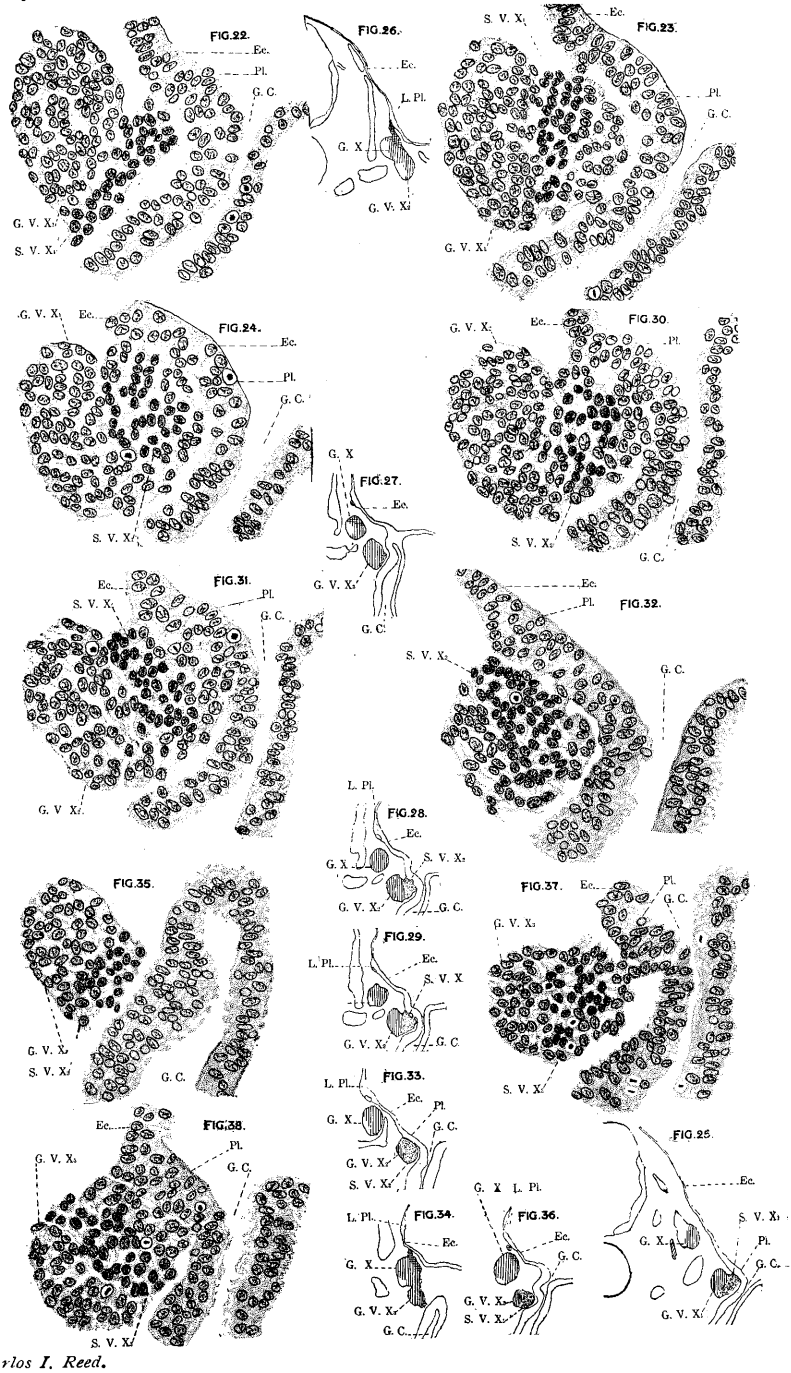
Fig. 34. Sec. No. 375. G. $X_3$. An outline drawing of a section anterior to the point of separation of the epibranchial ganglion from the main ganglionic mass of X; also anterior to the anterior extremity of the gill cleft, showing the lateralis component and a branch to the lateral line placode.

Fig. 35. Sec. No. 378. G. $X_3$. The outline of the general visceral component is fairly distinct and the mass of placodal cells relatively large; there is no contact with the ectoderm and it is impossible to distinguish the placode from the gill cleft thickenings.

Fig. 36. Sec. No. 380. G. $X_3$. The mass of contributed cells is very large but the placode is still indistinguishable from the gill cleft thickenings.

Fig. 37. Sec. No. 381. G. $X_3$. The general visceral portion of the ganglion is relatively small; there is evidence of active proliferation and a small area of contact but the extent of the placodal thickening is small.

Fig. 38. Sec. No. 383. G. $X_3$. The placodal thickening is quite marked and there is much evidence of active proliferation. The outline of the general visceral portion of the ganglion is quite distinct.

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## PLATE XXVI.

Fig. 39. Sec. No. 384. G. $X_3$. The mass of contributed cells is much larger in proportion to the size of the general visceral component, than in the preceding figure.

Fig. 40. Sec. No. 386. G. $X_3$. The placode is quite marked and there is active proliferation and an extensive contact area.

Fig. 41. No. 390. G. $X_3$. This section shows the placodal thickening persisting, even to the posterior extremity of the ganglion, where there is only a slight area of contact.

Fig. 42. Sec. No. 410. G. $X_4$. The apparently large size of the mass of contributed cells is probably exaggerated as a result of the peculiar form of the ganglion at this point. There is evidence of active proliferation but only a short area of contact.

Fig. 43. Sec. No. 410. G. $X_4$. An outline drawing of the same section as shown in Fig. 42, showing the relation of the placode to other structures. This magnification does not reveal the contact.

Fig. 44. Sec. No. 412. G. $X_4$. Showing the extensive contact and the large size of the mass of contributed cells.

Fig. 45. Sec. No. 413. G. $X_4$. The placode is quite distinct from the other ectodermal thickenings.

Fig. 46. Sec. No. 413. G. $X_4$. A high power drawing of the same section as shown in the preceding figure, showing evidence of active proliferation. The outline of the general visceral ganglion is fairly distinct.

Fig. 47. Sec. No. 415. G. $X_4$. Contact between the placode and the ganglion no longer exists but the placode is very large.

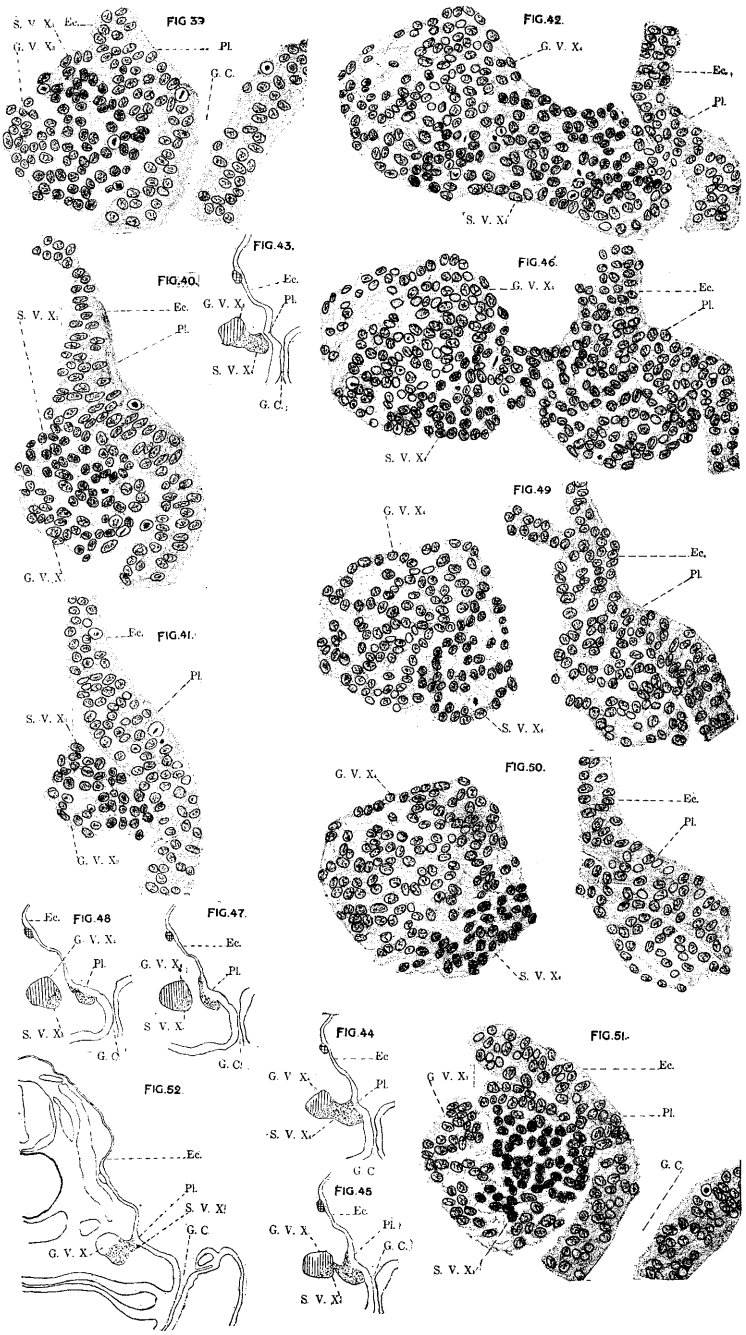
Fig. 48. Sec. No. 418. G. $X_4$. Showing partial detachment by lamellation, of the placodal mass from the ectoderm.

Fig. 49. Sec. No. 415. G. $X_4$. A high power drawing of the same section as shown in Fig. 47, showing a large mass of cells contributed by the placode but not yet metamorphosed. The outline of the general visceral component is fairly distinct and the nuclei of the placodal cells are slightly smaller than those of the general cells.

Fig. 50. Sec. No. 420. G. $X_4$. The placodal component is relatively small and a portion of the placodal mass is partially detached from the ectoderm by lamination.

Fig. 51. Sec. No. 329. G. $X_1$. A section of the first division of Gang. X, three sections posterior to that shown in Fig. 24.

Fig. 52. Sec. No. 428. G. X. Showing the general topography and the posterior extension of the main ganglionic mass of Gang. X, as it comes into contact with the ectoderm in the post-branchial groove.

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